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(54) Title: UROTENSIN-II AGONISTS AND ANTAGONISTS

(57) Abstract: The present invention features a novel class of cyclic polypeptides that have U-II antagonist and agonist activity. The invention also features methods for treating physiological or psychological conditions characterized by an excess or under expression of Ur4otensin-II.

UROTENSIN-II AGONISTS AND ANTAGONISTS

Field of the Invention

The invention relates to urotensin-II polypeptide agonists and antagonists and methods of their use.

Background of the Invention

Urotensin-II (U-II) is a cyclic neuropeptide with potent cardiovascular effects. Originally isolated from the caudal neurosecretory system of teleost fish, the primary structure of U-II has been established for several species of vertebrates, including various fish species, frogs, and humans. Sequence analysis of various U-II peptides from different species has revealed that, while the N-terminal region is highly variable, the C-terminal cyclic region of U-II is strongly conserved. Indeed, this cyclic region, which is responsible for the biological activity of U-II, is fully conserved from fish to humans (Coulouran, *et al.*, *Proc. Natl. Acad. Sci. USA* (physiology), 95:15803-15808 (1998)). The fact that evolutionary pressure has acted to fully conserve the biologically active sequence of U-II suggests that this polypeptide plays an important role in human physiology.

The cyclic region of U-II includes six amino acid residues (-Cys-Phe-Trp-Lys-Tyr-Cys- (SEQ ID NO: 1)) and is structurally similar to the biologically important central region of somatostatin-14 (-Phe-Trp-Lys-Thr- (SEQ ID NO: 2)). However, molecular cloning and sequence analysis of the carp preprourotensin II gene suggests that U-II and somatostatin are not derived from a common ancestor (Ohsako, S., *et al.*, *J. Neurosci.*, 6:2730-2735 (1986)).

In fish, U-II peptides have been shown to exhibit several activities, including general smooth muscle contracting activity, although responses vary between species and vascular beds (Davenport, A., and Maquire, J., *Trends in Pharmacological Sciences*, 21:80-82 (2000); Bern, H.A., *et al.*, *Recent Prog. Horm. Res.*, 45:533-552 (1995)). Fish U-II has also been shown to possess constrictor activity in mammals, including major arteries in rats, but the receptor(s) mediating these peptide actions are not fully characterized.

Recent studies have reported that an orphan human G-protein-coupled receptor, homologous to the rat GPR14 and expressed predominantly in cardiovascular tissue, functions as an U-II receptor (Ames, H., et al., *Nature*, 401:282-286 (1999)). Fish (goby) and human U-II reportedly bind to recombinant human GPR14 with high affinity, and the binding is functionally coupled to calcium mobilization. Human U-II is found within both vascular and cardiac tissue (including coronary atheroma) and effectively constricts isolated arteries from non-human primates (Ames, H., *et al., supra*). The potency of vasoconstriction of U-II is substantially greater than that of endothelin-1, making human U-II one of most potent mammalian vasoconstrictors currently known. *In vivo*, human U-II markedly increases total peripheral resistance in anaesthetized non-human primates, a response associated with profound cardiac contractile dysfunction (Ames, H., *et al., supra*).

Since human U-II-like immunoreactivity is found within cardiac and vascular tissue (including coronary atheroma), U-II is believed to influence cardiovascular homeostasis and pathology (*e.g.*, ischemic heart disease and congestive heart failure). Furthermore, the detection of U-II immunoreactivity within spinal cord and endocrine tissues suggests that U-II may have additional activities, including modulation of central nervous system and endocrine

function in humans (Ames, H., *et al.*, *supra*). Indeed, a number of maladies have been potentially linked to an excess or an under expression of U-II activity, including ischemic heart failure, hypotension, portal hypertension, angina pectoris, variceal bleeding, myocardial infarction, ulcers, and certain psychological and neurological disorders. Thus, there is a strong need for the development of potent compounds capable of modulating U-II activity, including U-II inhibitors or antagonists.

Summary of the Invention

The present invention features a novel class of cyclic polypeptides that have U-II antagonist activity. The polypeptides of the invention are octapeptides having the general formula: $(R^1)_a$-AA^1-cyclo[AA^2-AA^3-AA^4-AA^5-AA^6-Cys]-AA^7-R^2 (Formula I), wherein AA^1 is the L isomer of an aromatic amino acid; AA^2 is the L or D isomer of Cys; AA^3 is an L isomer of an aromatic amino acid; AA^4 is the L or D isomer of Trp; AA^5 is the L or D isomer of Lys, N-Me-Lys, or Orn; AA^6 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid; AA^7 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid; R^1 is H, a lower alkyl, lower alkanoyl, or a lower acyl; a is 1 or 2; and R^2 is OH, OR^3, $N(R^3)_2$, or NHR^3, where R^3 is H, a lower alkyl, or arylalkyl; provided that the peptide is not Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-NH_2.

In a preferred embodiment, AA^2 and AA^4 are D-Cys and L-Trp, respectively.

In another preferred polypeptide, AA^1 is Cpa, AA^2 is D-Cys, AA^3 is Phe, AA^4 is Trp, AA^5 is Lys, AA^6 is Thr, and AA^7 is Val.

In a particularly preferred embodiment, the polypeptide is an octapeptide having the formula Cpa-c[D-Cys-Phe-Trp-Lys-Thr-Cys]-Val-NH_2.

The invention also provides a Urotensin-II agonist polypeptide, and variants thereof, having the formula Asp-c[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH.

The polypeptides of the present invention are capable of altering U-II activity and can affect the binding of U-II to a receptor. Thus, these polypeptides may be administered to a subject as a means for preventing or treating medical or psychological conditions characterized by an excess or deficiency or under expression of Urotensin-II activity. Such conditions include, but are not limited to, ischaemic heart disease, congestive heart failure, portal hypertension, variceal bleeding, hypotension, angina pectoris, myocardial infarction, ulcers, anxiety, schizophrenia, manic depression, delirium, dementia, mental retardation, and dyskinesias.

The present invention also provides pharmaceutical compositions that include a therapeutically effective amount of a polypeptide of Formula I in combination with a pharmaceutically acceptable carrier. Suitable carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The composition can be adapted for the mode of administration and can be in the form of a pill, tablet, capsule, spray, powder, or liquid.

Other features and advantages of the invention will be apparent from the following detailed description thereof, and from the claims.

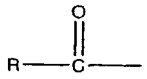
Definitions

By "polypeptide" is meant any peptide (including cyclic peptides) or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer

chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, or by chemical modification techniques which are well known in the art. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini.

The notations used herein for the polypeptide amino acid residues are those abbreviations commonly used in the art. The less common abbreviations Abu, Cpa, Nle, Pal, Tle, Dip, 4-Fpa, and Nal stand for 2-amino-butyric acid, p-chloroPhenylalanine, norleucine, 3-pyridyl-2-alanine, tert-leucine, 2,2-diphenylalanine, 4-fluoro-phenylalanine, and 3-(2-naphthyl)-alanine or 3-(1-naphthyl)-alanine, respectively

By "alkyl" is meant an aliphatic branched or straight chain hydrocarbon group. An alkyl is optionally substituted with one or more substituents which may be the same or different, and include, but are not limited to, halo, cycloalkyl, hydroxy, alkoxy, amino, carbamoyl, acylamino, aroylamino, carboxy, alkoxycarbonyl, aralkyloxycarbonyl, or heteroaralkyloxycarbonyl groups. Representative alkyl groups include, but are not limited to, methyl, trifluoromethyl, cyclopropylmethyl, cyclopentylmethyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, n-pentyl, 3-pentyl, methoxyethyl, and carboxymethyl. By "lower alkyl" is meant a branched or straight chain alkyl group having less than 11 carbon atoms, preferably a C_1-C_8 alkyl.

By "acyl" is meant a group having the structure  wherein R is H or an alkyl group as described herein. By "lower acyl" is meant an acyl group having less than 11 carbon atoms (either branched or straight chain), preferably between 1-8 carbon atoms (i.e., R is H or a lower alkyl).

By "lower alkanoyl" is meant an acyl group as described above wherein R is a lower alkyl.

By "aryl" is meant a monocyclic or bicyclic aromatic group containing from 6 to 12 carbons in the ring portion, preferably 6-10 carbons in the ring portion, such as phenyl, napthyl or tetrahydronaphthyl. By "arylalkyl" is meant an alkyl group as described herein having an aryl substituent, such as benzyl, phenylethyl or 2-naphthylmethyl.

By "pharmaceutically acceptable salt" is meant non-toxic acid addition salts or metal complexes which are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like.

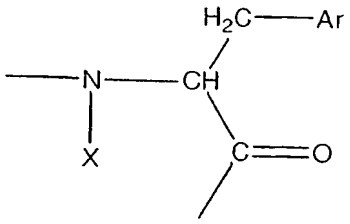
By "variant" is meant a polypeptide that differs from a reference polypeptide, but retains essential properties. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, and/or deletions, in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polypeptides may be made by mutagenesis techniques or by direct synthesis.

Generally, the variant differs from the reference polypeptide by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics (e.g. acidic, basic, aromatic, etc.). Typical substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.

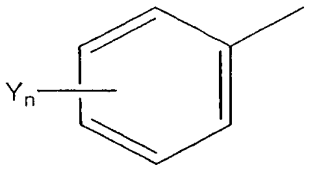
By "subject" is meant an animal or human suffering from a U-II-related physiological or psychological condition. The subject may be a mammal, including, but not limited to, humans and non-human mammals such as primates, dogs, cats, pigs, cows, sheep, goats, horses, rats, mice, and the like.

By "pharmaceutically acceptable carrier" is meant a carrier that is physiologically acceptable to an administered animal while retaining the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable carrier is physiological saline. Other physiologically acceptable carriers and their formulations are known to one skilled in the art and described, for example, in Remington's Pharmaceutical Sciences, (18th edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, PA.

By "aromatic amino acid" is meant an amino acid that contains an aromatic group. In preferred embodiments, the aromatic amino acid has the following formula:



(Formula II), where X represents a bond or H, and Ar is a moiety containing an optionally substituted aromatic ring. Examples of Ar, include but are not limited to, the following structures wherein Y_n represents n optional substituents and n is 0, 1, 2, or 3:



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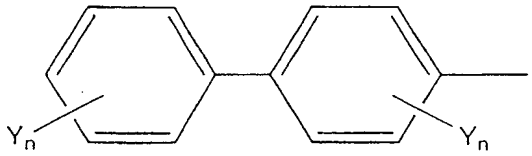

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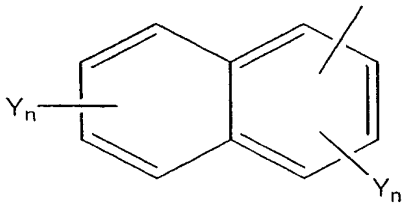

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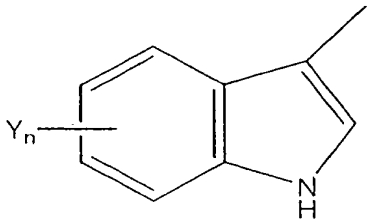

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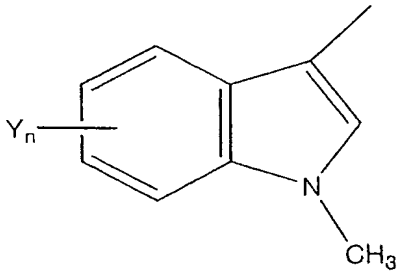
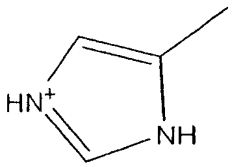


and



In preferred embodiments, each substituent Y independently represents NO_2, CN, Cl, Br, I, F, Me, COR^4, $COOR^4$, or OR^4, groups, where R^4 is H or C_1-C_8 alkyl. Examples of aromatic amino acids include, but are not limited to, Phe, Cpa, Trp, Pal, His, β-Nal, 3-pyridyl-Ala, 4-pyridyl-Ala, 2,4-dichloro-phe, pentafluoro-Phe, p-Z-Phe, and o-Z-Phe, wherein Z is selected from the group consisting of Me, Cl, Br, F, OH, OMe, and NO_2.

Detailed Description

We found that the minimum portion of the U-II sequence which retained full biological activity was the octapeptide Asp-c[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH (SEQ ID NO: 3), which corresponds to hUII(4-7). This octapeptide actually possess greater potency than the full human and fish U-II sequences in inducing rat aorta contraction and in binding to this tissue.

Based on this parent sequence, a series of cyclic octapeptides have been synthesized which have U-II antagonist activity. These peptides were discovered to have moderate affinity for U-II receptors and were able to block U-II-induced phasic contracts in circular rat thoracic aorta strips. The polypeptides of the present invention have the general formula: $(R^1)_a$-AA^1-cyclo[AA^2-AA^3-AA^4-AA^5-AA^6-Cys]-AA^7-R^2 (Formula I), wherein AA^1 is the L isomer of an aromatic amino acid; AA^2 is the L or D isomer of Cys; AA^3 is an L isomer of an aromatic amino acid; AA^4 is the L or D isomer of Trp; AA^5 is the L or D isomer of Lys, N-Me-Lys, or Orn; AA^6 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid; AA^7 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid; R^1 is H, a lower alkyl, lower alkanoyl, or a lower acyl; a is 1 or 2; and R^2 is OH, OR^3, $N(R^3)_2$, or NHR^3, where R^3 is H, a lower alkyl, or arylalkyl.

One of the most potent U-II inhibitors tested was the SRIF antagonist Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide (SEQ ID NO: 4), which had an IC_{50} of about 100 nM and Kd of 240. Another potent U-II antagonist was Cpa-c[D-Cys-Phe-Trp-Lys-Thr-Cys]-Val-NH_2 (SEQ ID NO: 5) which had an IC_{50} of about 2nM. Other SRIF antagonists that were tested are summarized in Example 2 below (see table 1).

The polypeptides of the invention are capable of modulating U-II activity and are, therefore, useful for treating physiological and psychological conditions related to either an excess of or an under expression of U-II activity within a subject. Such conditions include, for example, acute heart failure, hypotension, hypertension, angina pectoris, variceal bleeding, myocardial infarction, ulcers, and certain psychological and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, mental retardation and dyskinesias.

If the condition stems from an excess of U-II activity, one approach to treatment is to administer to a subject in need thereof an inhibitor compound (antagonist), optionally in combination with a pharmaceutically acceptable carrier, in an amount effective to inhibit the function of U-II. Alternatively, for treating conditions related to under expression of U-II activity, a compound which activates U-II (agonist) is administered.

A therapeutically effective amount of a polypeptide of Formula I, or a variant or pharmaceutically acceptable salt-thereof, can be administered orally, parenterally (e.g. intramuscular, intraperitoneal, intravenous or subcutaneous injection, or implant), nasally, vaginally, rectally, sublingually or topically, in admixture with a pharmaceutically acceptable carrier adapted for the route of administration.

Methods well known in the art for making formulations are found, for example, in Remington's Pharmaceutical Sciences (18th edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, PA. Compositions intended for oral use may be prepared in solid or liquid forms according to any method known to the art for the manufacture of pharmaceutical compositions. The compositions may optionally contain sweetening, flavoring, coloring, perfuming, and/or preserving agents in order to provide a more palatable preparation. Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier or excipient. These may include, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, sucrose, starch, calcium phosphate, sodium phosphate, or kaolin. Binding agents, buffering agents, and/or lubricating agents (e.g., magnesium stearate) may also be used. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and soft gelatin capsules. These forms contain inert diluents commonly used in the art, such as water or an oil medium. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying agents, and suspending agents.

Formulations for parenteral administration include sterile aqueous or non- aqueous solutions, suspensions, or emulsions. Examples of suitable vehicles include propylene glycol, polyethylene glycol, vegetable oils, gelatin, hydrogenated naphalenes, and injectable organic esters, such as ethyl oleate. Such formulations may also contain adjuvants, such as preserving, wetting, emulsifying, and dispersing agents. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene

copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for the polypeptides of the invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes.

Liquid formulations can be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, or by irradiating or heating the compositions. Alternatively, they can also be manufactured in the form of sterile, solid compositions which can be dissolved in sterile water or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to active substances, excipients such as coca butter or a suppository wax. Compositions for nasal or sublingual administration are also prepared with standard excipients known in the art. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops or spray, or as a gel.

The amount of active ingredient in the compositions of the invention can be varied. One skilled in the art will appreciate that the exact individual dosages may be adjusted somewhat depending upon a variety of factors, including the polypeptide being administered, the time of administration, the route of administration, the nature of the formulation, the rate of excretion, the nature of the subject's conditions, and the age, weight, health, and gender of the patient. In addition, the severity of the U-II-related condition being treated will also have an impact on the dosage level. Generally, dosage levels of between 0.1 μg/kg to 100 mg/kg of body weight are administered daily as a

single dose or divided into multiple doses. Preferably, the general dosage range is between 250 μg/kg to 5.0 mg/kg of body weight per day. Wide variations in the needed dosage are to be expected in view of the differing efficiencies of the various routes of administration. For instance, oral administration generally would be expected to require higher dosage levels than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, which are well known in the art. In general, the precise therapeutically effective dosage will be determined by the attending physician in consideration of the above identified factors.

The polypeptides of the invention can be administered in a sustained release composition, such as those described in, for example, U.S. Patent Nos. 5,672,659 and 5,595,760. The use of immediate or sustained release compositions depends on the type of condition being treated. If the condition consists of an acute or over-acute disorder, a treatment with an immediate release form will be preferred over a prolonged release composition. Alternatively, for preventative or long-term treatments, a sustained released composition will generally be preferred.

Polypeptides of the present invention can be prepared in any suitable manner. The polypeptides may be isolated from naturally occurring sources, recombinantly produced, or produced synthetically, or produced by a combination of these methods. The synthesis of short peptides is well known in the art. See e.g. Stewart *et al.*, Solid Phase Peptide Synthesis (Pierce Chemical Co., 2d ed., 1984). The peptides of the present invention can be synthesized according to standard peptide synthesis methods known in the art and exemplified in Example 1 below.

The present invention is illustrated by the following examples, which are in no way intended to be limiting of the invention.

Example 1 : Preparation of Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide

Step 1: Preparation of Boc-4-chlorophenylalanine-S-methylbenzyl-D-cysteine-3-pyridyl-2-alanine-D-tryptophan-N^{ε}-benzyloxycarbonyl-lysine-valine-S-methylbenzyl-cysteine-4-chlorophenylalanine-benzhydrylamine resin.

Benzhydrylamine-polystyrene resin (Advanced ChemTech, Inc., Louisville, KY) (1.2 g, 0.5 mmole) in the chloride ion form was placed in the reaction vessel of an Advanced ChemTech peptide synthesizer (Model 200) programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 min. and 25 min. each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; (f) 10% triethylamine in chloroform.

The neutralized resin is stirred with Boc-4-chlorophenylalanine and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 hour and the resulting amino acid resin in then cycled through steps (a) through (f) in the above wash program. The following amino acids (1.5 mmole) are then coupled successively by the same procedure: Boc-S-methylbenzyl-Cys, Boc-Val, Boc-N^{ε}-benzyloxycarbonyl-lysine, Boc-D-Trp, Boc-Pal, and Boc-S-methylbenzyl-D-Cys and Boc-4-chlorophenylalanine. After washing and drying, the completed resin weighed about 2.0 g.

Step 2: Deprotection and cleavage from resin.

The resin described in Step 1 (1.0 g, 0.25 mmole) was mixed with anisole (5 ml), dithiothretol (100 mg), and anhydrous hydrogen fluoride (35 ml) at about 0°C and stirred for 45 min. Excess hydrogen fluoride was evaporated rapidly under a stream of dry nitrogen, after which free peptide was precipitated and washed with ether. The crude peptide was then dissolved in 500 ml of 90% acetic acid. A concentrated solution of I_2/MeOH was then added until a permanent brown color was observed. Excess I_2 was removed by the addition of ascorbic acid and the solution evaporated to a small volume which was applied to a column (2.5 x 90cm) of VYDAC™ octadecylsilane silica (10-15μm). This was eluted with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid in water. Fractions were examined by thin layer chromatography and analytic high performance liquid chromatography and pooled to give maximum purity. Repeated lyophilization of the solution from water gave 125 mg of the desired product as a white, fluffy powder.

The product was found to be homogenous by HPLC and TLC. Amino acid analysis of an acid hydrolysate and matrix-assisted laser desorption MS confirmed the composition of the octapeptide. Other peptide of the invention may be made using an analogous procedure with appropriate reactants.

Example 2: Use of Rat Aorta Circular Strip for Assay U-II Antagonists

Male Sprague-Dawley rats (250-350 g), which had been quarantined for 5-7 days prior to the experiments, were sacrificed by decapitation (experiments were approved by the Advisory Committee For Animal Resources, Tulane University School of Medicine). The thoracic aorta was dissected, freed from connective tissue, and cut into rings of about 1.5 mm in width. The rings were

suspended in a 15 ml organ bath containing high potassium Kreb's solution (9.15 g/L potassium chloride, 2.1 g/L sodium bicarbonate, 1.0 g/L glucose, 0.16 g/L potassium phosphate monobasic, 0.14 g/L magnesium sulfate (anhydr.), and 0.22 g/L calcium chloride (dihydr.))

Optimal tension was applied (0.2 g) to the tissues and the bath medium was maintained at 37°C and bubbled with a mixture of 95% O_2/5% CO_2. Prior to mounting in the organ bath, selected preparations were rubbed with a moistened cotton wool swab, in order to remove the endothelial cell layer, and the effect of this procedure was tested using an acetylcholine-relaxation test. (Gibson, A., *Br. J. Pharmacol.* 91:205 (1987)). The aorta rings were allowed to equilibrate for 90 min. at the optimal tensions. During the equilibration period, the bath solution was replaced every 15 min. Contractile responses of aortae rings to various concentrations of peptides were expressed in volts. Changes in arterial smooth muscle tension were recorded isometrically using a force-displacement transducer (Radnoti), and a AcqKnowledge ACK100 Version 3.2 (BIOPAC Systems, Inc., Santa Barbara, CA.)

In siliconized glass tubes, peptides were dissolved in dionized water at a concentration of 1 μg/1μL (stock solution) and then diluted 1:10 with sterile BSA-saline solution (0.1% BSA, fraction V, Sigma, St. Louis in 0.9% NaCl). All peptide solutions were prepared fresh directly before the experiments. Peptides in the concentration ranges of 10^{-6} to 10^{-12} M/L in a final volume of 16-80 μL were direcly introduced into the tested organ bath containing Krebs buffer continuously gassed with 95% O_2 and 5% CO_2, and the aorta rings at an optimal resting tension (1 - 0.2g). Peptide-induced changes in tension of the aorta rings were recorded by force-displacement transducers and processed by the computer system BIOPAC Inc., as described above. Each ring was exposed to one peptide concentration only.

Using assay techniques known in the art, we found that the minimally, fully potent sequence of U-II was the octapeptide Asp-c[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH (SEQ ID NO: 3), which was actually more potent than the full human and fish sequences in inducing rat aorta contracts. Various somatostatin (SRIF) antagonists were discovered to have the ability to block UII-induced phase contractions in the circular rat thoracic aorta strips. One of the most potent inhibitors was the SRIF antagonist Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide (SEQ ID NO: 4), which had an IC_{50} of about 100 nM and a Kd of 240 nM. The polypeptide Cpa-c[D-Cys-Phe-Trp-Lys-Thr-Cys]-ValNH_2 was also a strong U-II antagonist with an IC_{50} of 2nM. Other compounds that were tested are summarized in Table 1 below.

Table 1. SRIF Antagonist IC_{50}s (nM) against U-II Stimulation of Rat Aorta Phasic Contractions

Polypeptide	IC_{50}
Nal-D-Cys-His-D-Trp-Lys-Val-Cys-D-Dip-NH_2 (SEQ ID NO: 6)	1800
4Fpa-D-Cys-Pal-D-Trp-Lys-Val-Cys-Nal-NH_2 (SEQ ID NO: 7)	1090
4Fpa-D-Cys-Pal-D-Trp-Lys-Tle-Cys-Nal-NH_2 (SEQ ID NO: 8)	100
Cpa-D-Cys-Tyr-D-Trp-Lys-Thr-Cys-Nal-NH_2 (SEQ ID NO: 9)	12
Cpa-D-Cys-Pal-D-Trp-Lys-Tle-Cys-Nal-NH_2 (SEQ ID NO: 10)	10
Cpa-D-Cys-Pal-Trp-Lys-Thr-Cys-Cpa-NH_2 (SEQ ID NO: 11)	2

Equivalents

Although the present invention has been described with reference to preferred embodiments, one skilled in the art can easily ascertain its essential characteristics and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various

usages and conditions. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed in the scope of the present invention.

All publications and patents mentioned in this specification are herein incorporated by reference.

What is claimed is:

1. A polypeptide or a variant thereof, said polypeptide having the formula:

$(R^1)_a$-AA^1-cyclo[AA^2-AA^3-AA^4-AA^5-AA^6-Cys]-AA^7-R^2

wherein

AA^1 is the L isomer of an aromatic amino acid;

AA^2 is the L or D isomer of Cys;

AA^3 is an L isomer of an aromatic amino acid;

AA^4 is the L or D isomer of Trp;

AA^5 is the L or D isomer of Lys, N-Me-Lys, or Orn;

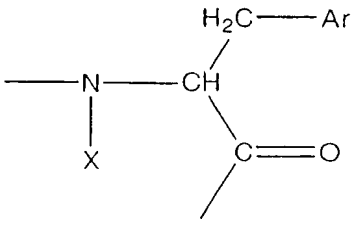
AA^6 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid;

AA^7 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid;

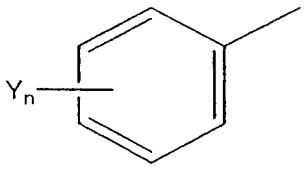
R^1 is H, lower alkyl, lower alkanoyl, or a lower acyl; a is 1 or 2; and R^2 is OH, OR^3, $N(R^3)_2$ or NHR^3, where R^3 is H, a lower alkyl, or arylalkyl; provided said peptide is not Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-NH_2; or

a pharmaceutically acceptable salt of said polypeptide or variant.

2. The polypeptide of claim 1, wherein said aromatic amino acid has the formula:



wherein X is H or a bond, and Ar represents a moiety selected from the group consisting of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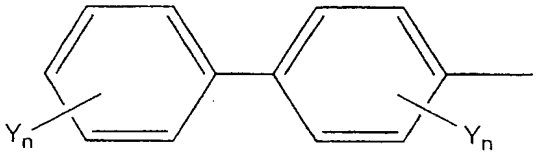
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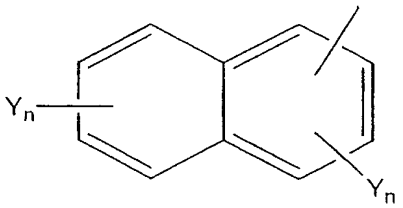
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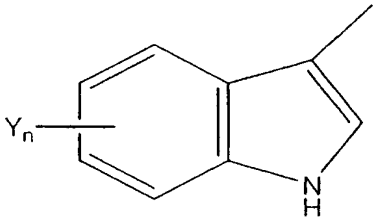
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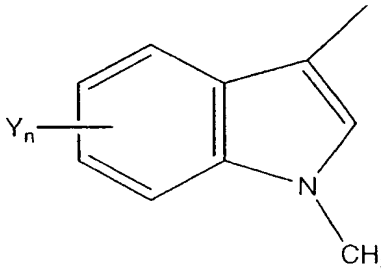
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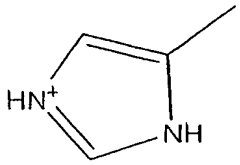


wherein n is 0, 1, 2, or 3 and each substituent Y independently represents NO_2, CN, Cl, Br, I, F, Me, COR^4, $COOR^4$, or OR^4, groups, where R^4

is H or C_1-C_8 alkyl.

3. The polypeptide of claim 1, wherein AA^3 is selected from the group consisting of Phe, Trp, Pal, His, β-Nal, 3-pyridyl-Ala, 4-pyridyl-Ala, 2,4-dichloro-phe, pentafluoro-Phe, p-Z-Phe, and o-Z-Phe, wherein Z is selected from the group consisting of Me, Cl, Br, F, OH, OMe, and NO_2.

4. The polypeptide of claim 1, wherein AA^4 is L-Trp.

5. The polypeptide of claim 1, wherein AA^2 is D-Cys.

6. The polypeptide of claim 5, wherein AA^3 is Phe, AA^4 is Trp, AA^5 is Lys, AA^6 is Thr, AA^7 is Val, and AA^1 is Cpa.

7. The polypeptide of claim 6, wherein said polypeptide has the formula Cpa-c[D-Cys-Phe-Trp-Lys-Thr-Cys]-Val-NH_2 (SEQ ID NO: 5).

8. A pharmaceutical composition comprising a polypeptide, or a variant thereof, and a pharmaceutically acceptable carrier, said polypeptide having the formula:

$(R^1)_a$-AA^1-cyclo[AA^2-AA^3-AA^4-AA^5-AA^6-Cys]-AA^7-R^2

wherein

AA^1 is the L isomer of an aromatic amino acid;

AA^2 is the L or D isomer of Cys;

AA^3 is an L isomer of an aromatic amino acid;

AA^4 is the L or D isomer of Trp;

AA^5 is the L or D isomer of Lys, N-Me-Lys, or Orn;

AA^6 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid;

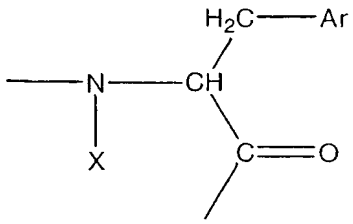
AA^7 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid;

R^1 is H, lower alkyl, lower alkanoyl, or a lower acyl; a is 1 or 2; and R^2 is OH, OR^3, $N(R^3)_2$ or NHR^3, where R^3 is H, a lower alkyl, or arylalkyl;

provided said peptide is not Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-NH_2; or

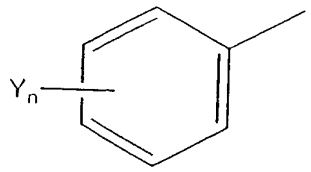
a pharmaceutically acceptable salt of said polypeptide or variant.

9. The pharmaceutical composition of claim 8, wherein said aromatic amino acid has the formula:



wherein X is H or a bond, and Ar represents a moiety selected from the group

consisting of



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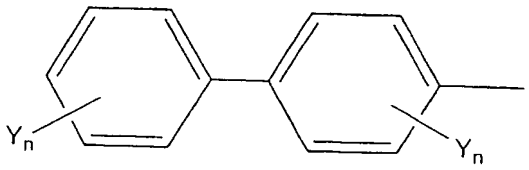

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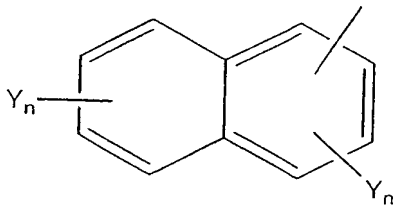

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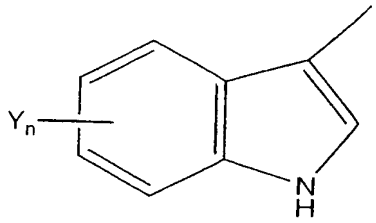

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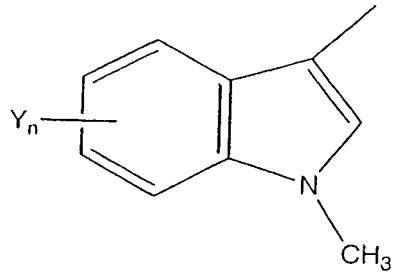

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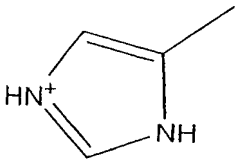

wherein n is 0, 1, 2, or 3 and each substituent Y independently represents NO_2, CN, Cl, Br, I, F, Me, COR^4, $COOR^4$, or OR^4, groups, where R^4 is H or C_1-C_8 alkyl.

10. The pharmaceutical composition of claim 8, wherein AA^3 is selected from the group consisting of Phe, Trp, Pal, His, β-Nal, 3-pyridyl-Ala, 4-pyridyl-Ala, 2,4-dichloro-phe, pentafluoro-Phe, p-Z-Phe, and o-Z-Phe, wherein Z is selected from the group consisting of Me, Cl, Br, F, OH, OMe, and NO_2.

11. The pharmaceutical composition of claim 8, wherein AA^4 is L-Trp.

12. The pharmaceutical composition of claim 8, wherein AA^2 is D-Cys.

13. The pharmaceutical composition of claim 12, wherein AA^3 is Phe, AA^4 is Trp, AA^5 is Lys, AA^6 is Thr, AA^7 is Val, and AA^1 is Cpa.

14. The pharmaceutical composition of claim 13, wherein said polypeptide has the formula Cpa-c[D-Cys-Phe-Trp-Lys-Thr-Cys]-Val-NH_2 (SEQ ID NO: 5).

15. The pharmaceutical composition of claim 8, wherein said carrier is selected from the group consisting of saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

16. A method of preventing or treating an abnormal condition characterized by an excess of Urotensin-II activity, said method comprising administering to a subject a therapeutically effective amount of a polypeptide, or variant thereof, said polypeptide having the formula:

$(R^1)_a$-AA^1-cyclo[AA^2-AA^3-AA^4-AA^5-AA^6-Cys]-AA^7-R^2

wherein

AA^1 is the L isomer of an aromatic amino acid;

AA^2 is the L or D isomer of Cys;

AA^3 is an L isomer of an aromatic amino acid;

AA^4 is the L or D isomer of Trp;

AA^5 is the L or D isomer of Lys, N-Me-Lys, or Orn;

AA^6 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid;

AA^7 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid;

R^1 is H, lower alkyl, lower alkanoyl, or a lower acyl; a is 1 or 2; and R^2 is OH, OR^3, $N(R^3)_2$ or NHR^3, where R^3 is H, a lower alkyl, or arylalkyl; or a pharmaceutically acceptable salt thereof.

17. The method of claim 16, wherein said condition is selected from the group consisting of ischaemic heart disease, congestive heart failure, portal hypertension, variceal bleeding, hypotension, angina pectoris, myocardial infarction, ulcers, anxiety, schizophrenia, manic depression, delirium, dementia, mental retardation, and dyskinesias.

18. The method of claim 17, wherein said condition is ischaemic heart disease.

19. The method of claim 17, wherein said condition is congestive heart failure.

20. The method of claim 17, wherein said condition is portal hypertension

21. The method of claim 17, wherein said condition is variceal bleeding.

22. A method of modulating the effect of a Urotensin-II (U-II) peptide, said method comprising administering to a subject a polypeptide, or variant thereof, said polypeptide having the formula:

$(R^1)_a$-AA^1-cyclo[AA^2-AA^3-AA^4-AA^5-AA^6-Cys]-AA^7-R^2

wherein

AA^1 is the L isomer of an aromatic amino acid;

AA^2 is the L or D isomer of Cys;

AA^3 is an L isomer of an aromatic amino acid;

AA^4 is the L or D isomer of Trp;

AA^5 is the L or D isomer of Lys, N-Me-Lys, or Orn;

AA^6 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid;

AA^7 is the L or D isomer of Val, Thr, Leu, Ile, tert-Leu, Abu, Nle, or an aromatic amino acid;

R^1 is H, lower alkyl, lower alkanoyl, or a lower acyl; a is 1 or 2; and R^2 is OH, OR^3, $N(R^3)_2$ or NHR^3, where R^3 is H, a lower alkyl, or arylalkyl; or

a pharmaceutically acceptable salt thereof.

23. The method of claim 22, wherein said modulating comprises decreasing the effect of said U-II peptide.

24. A urotensin II agonist polypeptide, or variant thereof, said polypeptide having the formula: Asp-c[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH (SEQ ID NO: 3).

25. A method of modulating the effect of a Urotensin-II (U-II) peptide, said method comprising administering to a subject the polypeptide of claim 24.

26. The method of claim 25, wherein said modulating comprises increasing the effect of said U-II peptide.

27. A method of preventing or treating an abnormal condition characterized by an under expression of Urotensin-II activity, said method comprising administering to a subject a therapeutically effective amount of the polypeptide of claim 24.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(30) Priority Data:
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Filed on 20 October 2000 (20.10.2000)

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:
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4 September 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/032932 A3

(54) Title: UROTENSIN-II AGONISTS AND ANTAGONISTS

(57) Abstract: The present invention features a novel class of cyclic polypeptides that have U-II antagonist and agonist activity. The invention also features methods for treating physiological or psychological conditions characterized by an excess or under expression of Ur4otensin-II.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/50724

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/575 A61K38/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOCART SJ ET AL.: "Highly Potent Cyclic Disulfide Antagonists of Somatostatin" JOURNAL OF MEDICINAL CHEMISTRY, vol. 42, no. 11, 3 June 1999 (1999-06-03), pages 1863-1871, XP002189091 the entire document, in particular tables 1 and 2, page 1864, left-hand column and page 1868, right-hand column	1-3,5, 8-10,12, 15-23
X	HOCART SJ ET AL.: "Potent Antagonists of Somatostatin: Synthesis and Biology" JOURNAL OF MEDICINAL CHEMISTRY, vol. 41, 26 March 1998 (1998-03-26), pages 1146-1154, XP000749590 page 1146; tables 1,2	1-3,5, 8-10,12, 15-23

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 November 2002	29/11/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schmidt, H

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	NISHIKI M ET AL.: "Histopathological Improvement of Acromegalic Cardiomyopathy by Intermittent Subcutaneous Infusion of Octreotide" ENDOCRINE JOURNAL, vol. 44, no. 5, 1997, pages 655-660, XP008010820 abstract	16-23
A	NEWBY DE & JALAN R: "Urotensin II: Better than somatostatin for portal hypertension?" HEPATOLOGY, vol. 31, no. 5, May 2000 (2000-05), pages 1201-1202, XP008010830 page 1201	16-23
A	FR 2 786 489 A (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE INSERM) 2 June 2000 (2000-06-02) the entire document, in particular SEQ ID NOs 3 and 9, page 8 and figure 1	1-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/50724

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 16-23 and 25-27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-27 (all partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-27 (all partially)

The scope of claims 1-27, in as far as the expression "variant" is concerned, is so unclear (Article 6 PCT) that a meaningful search was impossible with regard to this expression.
Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the polypeptides as such within the meaning of claim 1.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr[redacted]nal Application No
PCT/US 01/50724

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		EP 1131436 A1	12-09-2001
		WO 0031265 A1	02-06-2000
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